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Short Communication

Species-specific impact of protists in controlling litter decomposition

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ABSTRACT

Protists affect soil microbiome composition and functioning, potentially increasing litter decomposition and plant growth. Yet, the role of different protist species, individually or in combinations, in regulating microbial-mediated litter decomposition remains unknown, as well as if these interactions feedback to plant growth. Using a full-factorial design of three protist species combinations with bacterial and fungal communities, we found that only one protist species reduced litter decomposition by 19%, with other species and combinations not affecting litter decomposition. Despite a positive correlation between plant growth and litter decomposition, we did not observe protist-induced changes in plant growth. Overall, our results highlight that protists affect litter decomposition in a species-specific manner, including reducing litter decomposition.

Litter decomposition plays a vital role in soil organic matter formation, nutrient cycling, biodiversity conservation, and other ecosystem functions (Swift et al., 1979). For example, nutrients released during litter decomposition can promote plant growth (Chapman et al., 2006). Litter decomposition is mainly regulated by three main factors: the physicochemical environment, the litter's physicochemical properties, and soil biota (Coûteaux et al., 1995). Among soil biota, fungi are the dominant decomposers (Zhang et al., 2024). Fungi (and other microorganisms) are not only influenced by substrate characteristics from bottom-up processes (Sinsabaugh et al., 2009) but also by top-down predation, such as by protists (Thakur and Geisen, 2019). While suggested to be predominantly bacterivores (De Ruiter et al., 1995), protists are increasingly shown to feed on fungi and shape fungal communities (Geisen et al., 2016). Thereby, and by indirectly shifting bacterial-fungal dominance with importance for ecosystem functions (Bahram et al., 2018), protists likely impact litter decomposition and, subsequently, plant growth. For example, microbial predation by the model protist *Physarum polycepharum* increases litter decomposition (Geisen et al., 2021). Additionally, faster litter decomposition enhances nutrient cycling, increasing the likelihood of positive plant-soil feedback (Kardol et al., 2015). Yet, each protist species from the incomparably high pool of phylogenetically distinct units possesses unique traits, resulting in divergent feeding patterns (Bell et al., 2010; Geisen et al., 2016; Estermann et al., 2023), potentially differentially affecting litter decomposition. Species-specific trait differences further suggest that interactions of co-existing protist species – the default in all soils – might affect overall community functioning (Saleem et al., 2012), such as through complementary effects (Ives et al., 2005). However, the knowledge of protists on litter decomposition is limited to a single model species (Geisen et al., 2021), with missing insights into protist species-specific differences and potential interactive effects on litter decomposition and if these effects alter plant growth.

To address the effects of different protist species and combinations on litter decomposition and resulting consequences for plant growth, we conducted experiments using three phylogenetically diverse amoeboid protists (*Didymium* sp. (Amorphea); *Cercomonas ambigua* (TSAR); *Tetramitus thorntoni* (Excavates) in a full factorial design including all possible combinations, along with either bacteria alone or bacteria & fungi (Fig. S1). We first carried out a 40-day litter decomposition experiment using commercially available green tea (EAN 8787113275157 27; Lipton, Unilever) as litter substrate (Keuskamp et al., 2013) to examine the effects of different protist treatments on decomposition. Subsequently, we used the remaining litter residue to set up a 35-day plant growth experiment with *Brassica napus* as a model plant species to address the effects of protist-mediated decomposition on plant growth (see supplementary material for more details). We hypothesized that 1) protists generally increase litter decomposition, with the magnitude depending on protist species identity; 2) protist species combinations increase the effects of single protist species on litter decomposition; and 3) protist-induced increases in litter decomposition

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will enhance plant growth.

We found that fungi mainly regulated litter decomposition (ANOVA; $F = 1563.2$, $p < 0.01$; Fig. S2), which confirms previous reports (Zhang et al., 2024). Therefore, we focused mainly on the bacteria & fungi treatment in the main text (see supplementary material and Fig. S3 for results of the bacteria-only treatment). In contrast to the first hypothesis and a previous study focusing on the protist model species *Physarum polycephalum*, protists generally did not increase litter decomposition (ANOVA; $F = 2.364$, $p = 0.130$; Fig. 1A). Several reasons could contribute to this inconsistency. First, environmental variations may play a role. In comparison to increased litter decomposition observed at 17 °C, *P. polycephalum* did not increase litter decomposition at 21 °C (Geisen et al., 2021), a temperature similar to our experiment running at 20 °C. Overall, temperature differences can shape the effect of larger soil predators on decomposition (Koltz et al., 2018). Second, differences in litter type may also contribute to the outcome of functional predator-prey changes. Geisen et al. (2021) used high lignin-containing litter (*Quercus robur*), while we used low lignin-containing litter (green tea). As previously shown, high-lignin litter shapes the microbial composition towards specialized taxa, while low-lignin litter material shapes it towards generalized taxa (Evans et al., 2017). These variations in microbial composition induced by litter type variations may lead to diverse responses to protist predation in litter decomposition. Third, the ability of *P. polycephalum* to directly consume organic material (Oettmeier, 2019) is not reported for the protist species studied here.

We also found protist species-specific differences in the impact on litter decomposition, with only one species, *T. thorntoni,* affecting litter decomposition, which also largely contradicted hypothesis 1. Trait variation might underlie these effects as previous studies highlighted that e.g. body size can determine prey composition (Gao, 2020). Thus, the smaller *Didymium* sp. and *C. ambigua* might not affect litter decomposition due to their smaller size than that of *T. thorntoni*, but also other unmeasured traits might differentiate their impact from that of *T. thorntoni.* In fact, rather than increasing litter decomposition as shown for *P. polycephalum* (Geisen et al., 2021), *T. thorntoni* decreased litter decomposition by 19.3% compared to the control (Tukey HSD, $p < 0.05$; Fig. 1B). The reduction of litter decomposition cannot be explained by *T. thorntoni* reducing the fungal concentration (Tukey HSD, $p = 0.536$; Fig. S4A), despite the overall trend of a positive correlation between fungal concentration and litter decomposition ($R = 0.25$, $p = 0.063$; Fig. S4B). Therefore, the mechanisms driving the reduction in litter decomposition by *T. thorntoni* remain to be discovered, potentially including inhibition of fungal activity, such as by promoting anti-fungal bacteria (Guo et al., 2022) or community changes disfavoring fungal decomposers.

In contrast to hypothesis 2, diverse protist species combinations did not increase the effects of single protist species on litter decomposition (Fig. 1B). This lack of complementary effects (Ives et al., 2005) is likely driven by the fact that two out of three species did not affect litter decomposition. Protists also did not alter plant growth (ANOVA; $F = 1.422$, $p = 0.233$; Fig. 2A), despite a positive link between plant growth and litter decomposition ($R = 0.27$, $p = 0.044$; Fig. 2B). Again, the limited general effects of protists on litter decomposition might explain the lack of potentially protist-induced feedback on plant growth (hypothesis 3). These findings suggest that while plants benefit from released nutrients (Chapman et al., 2006), the presence of specific protist species, at least when they do not alter litter decomposition as mostly found here, does not generally affect plant growth.

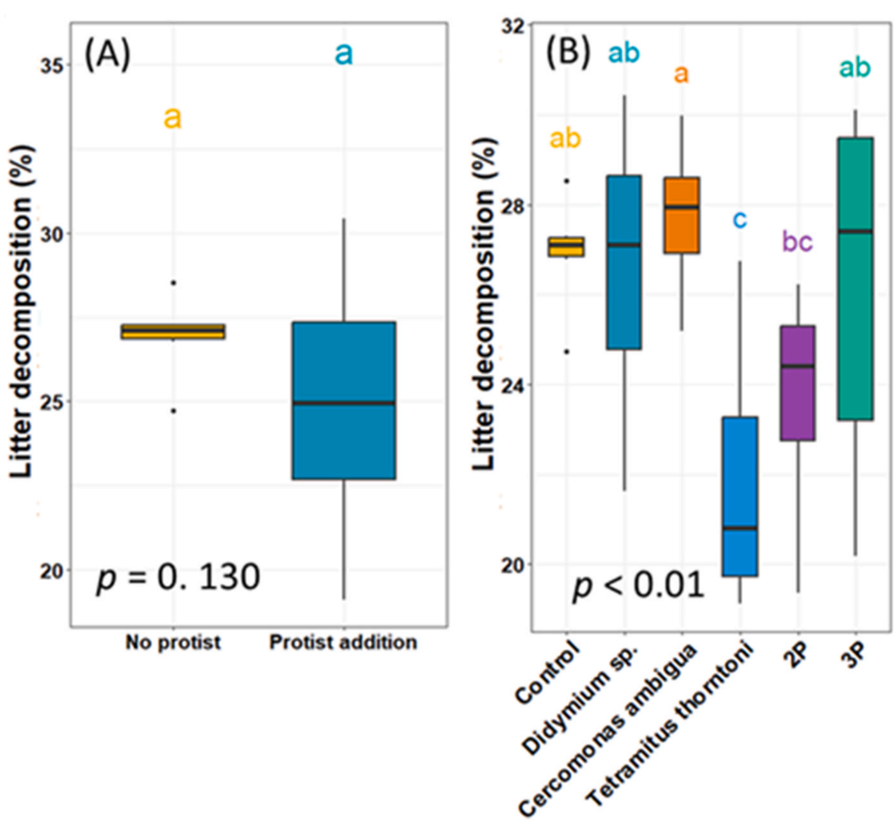

Fig. 1. Effects of overall protist addition (A) and different protist species and combinations (B) on litter decomposition in the bacteria & fungi treatment. Litter decomposition is represented by mass loss data, indicating the percentage of decomposed litter compared to its initial mass. Control = no protist addition, 2P includes all possible two-species combinations of the three protist species, in 3P, all three different protist species were combined; horizontal bars within boxes represent the median, with the tops and bottoms of boxes indicating the 75th and 25th quartiles, respectively. Whiskers depict the range of non-outlier data values, while outliers are plotted as individual points. Significant differences between different treatments are evaluated by ANOVA tests ($p < 0.05$), with different letters above bars indicating significant distinctions tested by LSD post-hoc tests ($p < 0.05$).

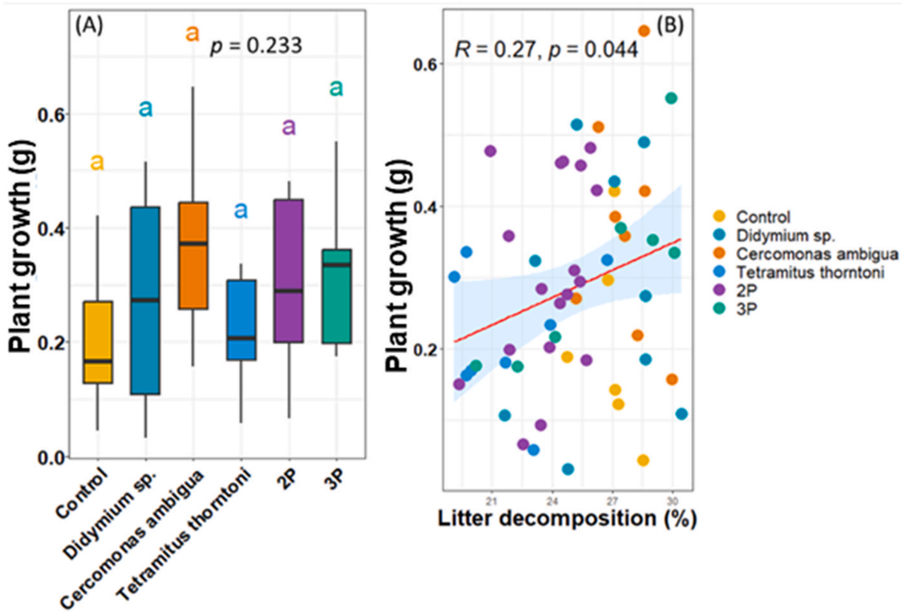

Fig. 2. Effects of different protist species and combinations on plant growth (A) and correlation between plant growth and litter decomposition (B) in the bacteria & fungi treatment. Litter decomposition is represented by litter mass loss data, indicating the percentage of decomposed litter compared to its initial mass. Plant growth is defined by the total dry mass of aboveground and belowground biomass (g). Control = no protists addition, 2P includes all possible two-species combinations of the three protist species; in 3P, all three different protist species were combined; in (A) horizontal bars within boxes represent the median, with the tops and bottoms of boxes indicating the 75th and 25th quartiles, respectively. Whiskers depict the range of non-outlier data values, while outliers are plotted as individual points. Significant differences between different treatments are evaluated by ANOVA tests ($p < 0.05$), with different letters above bars indicating significant distinctions tested by LSD post-hoc tests ($p < 0.05$).

In summary, only one - of three species of protists and their combinations reduced litter decomposition, with plant growth not being affected by protists. As our findings contradict previous suggestions, combined with the highly limited set of protist species so far linked to their impact on litter decomposition, further studies with additional protist species are needed to obtain a cumulative understanding of the role of protists on litter decomposition and for interpreting the outcomes of future integrative plant-soil feedback experiments. In particular, mechanisms underlying the role of protists in litter decomposition should be targeted, such as the role of interactions with bacteria and fungi, and how these will link to the dominance of litter decomposers (as we see a trend for a decrease in fungal concentration with the litter decomposition-reducing *T. thorntoni*). In addition, research efforts should focus on investigating if and which protist species might have a predominant role in regulating litter decomposition and on evaluating the extent of transferability between patterns found in simplified laboratory systems to field conditions, which involve more diverse factors such as litter types, soil types, and other physicochemical properties. These efforts will help to assess the overall direction protists have in the carbon cycle, the resulting feedback effects on plants (Kardol et al., 2015), and main factors influencing the direction of protist-induced litter decomposition.

CRediT authorship contribution statement

Yuxin Wang: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Thomas Edison E. dela Cruz:** Writing – review & editing, Supervision, Methodology, Conceptualization. **James Kennard S. Jacob:** Writing – review & editing, Methodology, Investigation. **Stefan Geisen:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2024.109598.

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